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A quick RNA mini-prep for Neurospora mycelial cultures

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Abstract

Most RNA isolation techniques currently in use have been developed for the processing of large quantities of material. These typically involve multiple phenol extractions (Reinert et al. 1981 Mol. Cell Biol. 1:829-836) or guanadinium isothio-cyanate/cesium chloride gradients (Chirgwin et al. 1979 Biochem 18:5294-5299) and can be both expensive and time consuming. Often, however, needs arise where quantitatively smaller amounts of RNA are needed from many different samples, for example, during time series analyses or when screening transformants for expression of a transformed gene. Under such circumstances, existing techniques are overly time consuming and yield more RNA than is necessary. The availability of a rapid RNA mini-prep is thus desirable. Such a system has been developed for isolating plant RNA (Nagy et al. 1988 Plant Molecular Biology Manual, B4; ed. Gelvin and Schilperoort, Klewer Academic Publishing, pp. 1-29), and we have adapted this procedure for use with Neurospora and, potentially, other filamentous fungi. Below, we describe the use of this procedure with 50 ml mycelial cultures, although we have used in with equal success with 5 ml cultures without scaling down the amounts of any reagents.

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A quick RNA mini-prep for Neurospora mycelial cultures

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Most RNA isolation techniques currently in use have been developed for the processing of large quantities of material. These typically involve multiple phenol extractions (Reinert et al. 1981 Mol. Cell Biol. 1:829-836) or guanadinium isothio-cyanate/cesium chloride gradients (Chirgwin et al. 1979 Biochem 18:5294-5299) and can be both expensive and time consuming. Often, however, needs arise where quantitatively smaller amounts of RNA are needed from many different samples, for example, during time series analyses or when screening transfor-mants for expression of a transformed gene. Under such circumstances, existing tech-niques are overly time consuming and yield more RNA than is necessary. The availability of a rapid RNA mini-prep is thus desirable. Such a system has been developed for isola-ting plant RNA (Nagy et al. 1988 Plant Molecular Biology Manual, B4; ed. Gelvin and Schilperoort, Klewer Academic Publishing, pp. 1-29), and we have adapted this procedure for use with Neurospora and, potentially, other filamentous fungi. Below, we describe the use of this procedure with 50 ml mycelial cultures, although we have used in with equal success with 5 ml cultures without scaling down the amounts of any reagents.

The method involves the use of a triphenylmethane dye, aurintricarboxylic (ATA), to protect the RNA. ATA binds irreversibly to RNA and is a potent inhibitor of most nucleic acid binding enzymes (Hallick et al. 1977 Nucl. Acids Res. 4:3055-3064). Thus, RNA made with procedure cannot be used for *in vitro* transcription or translation or reverse transcription but works fine for RNA/DNA or RNA/RNA hybridizations.

To minimize RNase contamination, all glassware is baked at 182°C for at minimum of four hours. Work with gloved hands. The procedure is as follows:

1. Conidia from slants (grown in 16 x 150 mm test tubes containing 8 ml of solid medium) are resuspended in 50 ml of Horowitz complete medium (Horowitz 1947 J. Biol. Chem. 171:255-262) and the cultures grown overnight with shaking at 30°C. A 50 ml culture typically yields enough RNA for 200 gel lanes (see below), and, as noted, smaller culture volumes may be used.

2. Flat mycelial pads are easier to grind than mycelial balls. Therefore, filter cultures using a Buchner funnel onto Whatman #44 filter paper. Wrap flat mycelial pads in aluminum foil and freeze in dry ice. Do not freeze in EtOH/dry ice bath because alcohol might seep through foil. Pads can be stored at -70°C for at least three weeks.

3. Wash a mortar and pestle thoroughly with warm water and Alconox (Fisher Scientific); cool by filling with liquid N_2. Remove frozen, flat mycelia from foil and add it to the liquid N_2 in mortar. Add ~0.5 g of baked sand and grind mycelial pad to a fine powder. Add more N_2 as needed. Mortar and pestle should be washed after every sample.

4. Working quickly before powder can thaw, pour or spoon ground mycelia into 15 ml round bottom Sarstedt tube (Sarstedt tubes #60.540, Princeton, NJ) containing 8 ml of E buffer at room

temperature. [E buffer: 50 mM Tris-Cl pH 8.0, 300 mM NaCl, 5 mM EDTA, pH 8.0, 2% SDS; autoclave and add 1 mM ATA and 14 mM ß-mercaptoethanol. ATA=aurintricarboxylic acid, ammonium salt (Sigma #A0885, St. Louis, MO)]

5. Thaw the powder in E buffer in 42°C water bath, occasionally shaking, to get SDS into solution. This should take about 5 minutes.

6. Add 1.1 ml of 3M KCl, invert to mix, keep on ice for 10 min. Solution should form semi-solid, flocculent mass as K-SDS precipitate forms.

7. Spin at 3000g, 4°C in a fixed angle rotor. Make sure caps are screwed on tightly to prevent tubes from collapsing.

8. Pass supernatant through 50 micron Miracloth (Calbiochem #475855, La Jolla, CA) in a funnel into fresh Sarstedt tube.

9. Measure volume of average-sized sample. Add 0.5 vol. 8 M LiCl, mix and stand at 4°C overnight.

10. Spin at 12000g, 4°C, for 15 min in a fixed angle rotor. Thoroughly resuspend pellet in 4 ml sterile gd (glass distilled) H_2O with pasteur pipette.

11. Extract twice with phenol/chloroform/isoamyl alcohol (25:24:1), spinning 12000g 10 min at 4°C in a fixed angle rotor. Save aqueous (upper) phase; add gd H_2O if volume is less than 2 ml.

12. Add 0.1 vol 3M NaOAc pH 6.0, mix and add 2.5 vol EtOH, mix. Place at -20°C overnight or -70°C for 15 min.

13. Spin 12000g, 10 min, 4°C. Wash pellet with 70% EtOH and drain. Pellet should be a light pink or white.

14. Resuspend in 0.4 ml sterile gd H_2O in a microfuge tube. Precipitate with NaOAc and EtOH as in step 12.

15. Spin 10-20 min in microfuge. Wash twice with 70% EtOH and dry pellet. Resuspend in 200 µl of sterile RNase-free gd H2O. Store at -70°C for up to three months. Spectophotometric quantification may be done at this point.

16. Load 1 µl onto a formaldehyde gel. Electrophorese overnight at 20 volts. Blot onto nitrocellulose. Probe with DNA fragment of choice. Expose to film.

Yields are typically on the order of 1-2 mg total RNA from an overnight 50 ml culture arising from an average slant. The number of samples able to be processed using this procedure is limited by the number of spaces in a centrifuge rotor. We have done as many as 24 samples in one day, and doing several times this many would be possible. We have observed on ethidium bromide stained gels that the fluorescence from the RNA deriving from this miniprep is brighter

than that seen when the corresponding amount of standard-prep RNA is used. This may be due to enhanced fluorescence of RNA in the presence of ATA. However, autoradiography of the blots does not show any RNA degradation products (Figure 1). This procedure would probably work fine with other methods of tissue disruption. ATA inhibits many nucleic acid binding proteins, possibly by competing for binding sites (Blumenthal and Landers 1973. BBRC 55:680-688). Therefore, the most critical factor is getting the RNA in contact with ATA before nucleases can bind to the nucleic acid and degrade it. Supported by federal grants to J.J.L. and J.C.D.

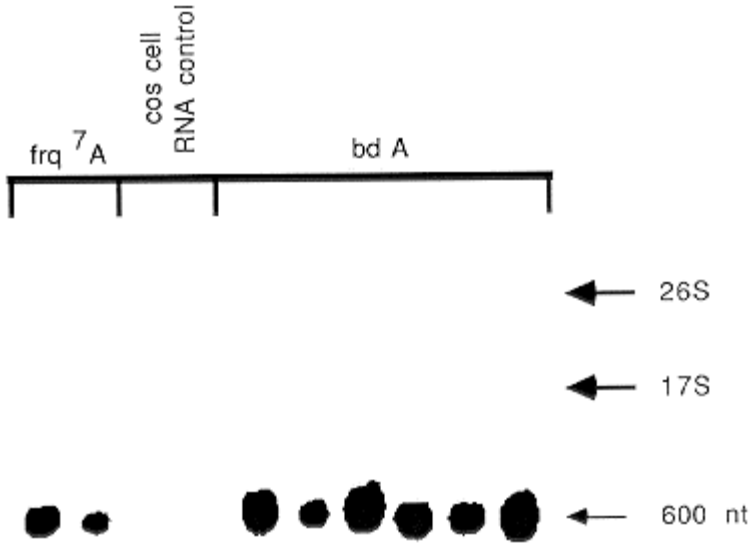

Fig. 1. ATA mini-prep RNA probed with *ccg-1* DNA fragment. Total RNA from a series of transformants into *bd A* and *frq7 A* was examined for the presence of the *ccg-1* gene transcript (Loros et al. 1989 Science 243:385-388). Each lane contains 10 µg of RNA (1/200 of the preparation). While the fluorescence staining of the RNA extended from the 26S to below the 17S RNA bands (not shown), the hybridization revealed the presence of only a single undegraded transcript in each lane containing transformant RNA and no hybridization to the monkey cos cell RNA control.